Arabidopsis F-box gene *FOA1* involved in ABA signaling

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The expression of *FOA1* (F-box overexpressed/oppressed ABA signaling) in different organs of *Arabidopsis*, and in response to ABA and NaCl, was analyzed. The expression level of *FOA1* is higher in the root and is lower in the stem, and is induced rapidly by ABA and NaCl. The phenotypes of T-DNA insertion mutant *foa1* and *FOA1* overexpression lines *FOA1ox1* and *FOA1ox2* were analyzed. The *foa1* mutant exhibited a lower germination rate, shorter root length, more stomatal opening, increased proline accumulation and hypersensitivity to ABA compared with the wild type. In contrast, the overexpression lines showed lower sensitivity to ABA than the wild type. The expression levels of several ABA and stress-responsive transcription factors and genes were altered in the *foa1* mutant in response to ABA. Compared with the wild type, the expression levels of ABA-responsive transcription factors were higher, but ABA and stress-responsive genes were lower in *foa1* mutant. This study demonstrates that *FOA1* is an ABA signaling-related gene, and may play a negative role in ABA signaling.

F-box gene, *FOA1*, ABA, signaling, *Arabidopsis*

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Protein degradation is an important post-transcriptional regulation process that permits organisms to quickly respond to a changing environment, either inside or outside the cell, by adjusting the quantity of key proteins. Most cellular proteins are degraded via the UPS (ubiquitin proteasome system) pathway [1,2]. Three types of enzymes, E1 (ubiquitin activating enzymes), E2 (ubiquitin conjugating enzymes) and E3 (ubiquitin ligases), are involved in the UPS pathway for ubiquitination and degradation of target proteins. E3s display substrate specificity, specifically recognizing target proteins and subsequently mediating their proteolysis. E3s have become a current research hot spot. There are about 1200 E3s in *Arabidopsis thaliana* [2–4], which can be classified into several large families. SCF (SKP1-CUL1-F-box) is one of the largest, best understood, most-characterized, and widely studied E3s [5,6].

As members of the SCF complex, F-box proteins are widely distributed among living organisms. They regulate many physiological phenomena, including hormone signal transduction (for example SLY1 and GID2 in Gibberellic acid (GA) signaling [7], EDL3 in abscisic acid (ABA) signaling [8], EBF1 and EBF2 in ethylene signaling [9], and TIR1 in auxin signaling [10]), self-incompatibility, floral development and circadian clock [11,12] through their specific recognition of particular substrates. In addition, F-box proteins are involved in plant abiotic stress responses [13–15], including to drought [16–19], cold [20] and high salt [18] stress. Studies of the function of F-box proteins and their related target proteins will aid in the in-depth understanding of plant growth and related signal transduction processes.

There are approximately 700 F-box proteins in *Arabidopsis* [1], but the functions of most of them remain unknown. *Arabidopsis* gene *AT3G17320* encoding an F-box protein, named for *FOA1* (F-box overexpressed/oppressed

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ABA signaling), is located on the third chromosome and has a length of 1230 bp and has no introns. The protein contains a typical F-box structure domain [1,21] and a conserved FBA_1 domain, which may act as a new protein-protein interaction motif [21].

A 1204 bp genomic DNA sequence of *AT3G17320* (−1201 to +3) was analyzed using PLACE software (<http://www.dna.affrc.go.jp/PLACE/>), and certain plant hormone and stress-response elements were found, including AuxRE (Auxin responsive element), GARE (Gibberellin responsive element), ABRE (ABA responsive element), and LTRE (low temperature responsive element). Thus, we speculated that the expression of *FOA1* gene is regulated by hormones and environmental stresses, and that it might play an important role in hormone and stress-response pathways. To reveal the biological function of *FOA1*, we analyzed the expression level of *FOA1* in different organs of *Arabidopsis* and in response to ABA and NaCl. Subsequently, T-DNA insertion mutant and over-expression lines of *FOA1* were used to investigate its role in ABA signaling.

1 Materials and methods

1.1 *FOA1* gene clone and plant transformation

Fragments of the *FOA1* gene with attB sites were amplified from wild-type *Arabidopsis* (Col-0) cDNA by polymerase chain reaction (PCR) using prime STAR high fidelity polymerase (TaKaRa, Japan) and primers *FOA1F* and *FOA1R* (Table 1; attB sites are underlined). The fragments were subcloned into Entry Vector pDONR/ZEO using BP Clonase II (Invitrogen, USA) and sequenced. Positive fragments were cloned into destination vector pEarleyGate 203 (N-Myc) using LR Clonase II (Invitrogen, USA), and then introduced into *Agrobacterium tumefaciens* GV3101 by electroporation and transformed into the *Arabidopsis thaliana* mutant *sgs* (suppressor of gene silencing) (kindly provided by Professor Lin ChenTao's laboratory from University of California, Los Angeles, USA) using the floral dip method [22]. The *sgs* mutant, developed using ethyl methanesulfonate, carries a recessive monogenic mutation that acts *in trans* to specifically impede transgene-induced post-transcriptional gene silencing [23]. Thus, it can be used to express transgenes, avoiding the co-suppression phenomenon. The transgenic plants were screened using the herbicide Basta to identify homozygous lines. Leaves from the homozygous lines were collected, frozen in liquid nitrogen, and stored at −80°C for RNA isolation and gene expression analysis.

1.2 Identification of T-DNA insertion mutants

T-DNA insertion mutant *CS857416* was purchased from the biological resources center (ABRC) and is in the Columbia

background (Col-0). Homozygous lines were identified by PCR using primers F, R, and the left boundary sequence of T-DNA, named R0, provided by ATIDB (the *Arabidopsis thaliana* Integrated Database) (Table 1). DNA was extracted from the leaves of T-DNA insertion mutants using the CTAB method and used as templates for PCR. Thereafter, the leaves of identified homozygous lines were collected, frozen in liquid nitrogen, and stored at −80°C for RNA isolation and gene expression analysis.

1.3 ABA and NaCl treatment, seed germination and root elongation assay

Seeds were surface-sterilized in 75% ethanol for 30 s, followed by 10% NaClO for 10 min, washed with sterile distilled water four to six times, and then stored in the dark at 4°C for 4 d. These seeds were used for the ABA and NaCl treatment, seed germination and root elongation assay.

ABA treatment was as follows: Seeds were suspended in 0.1% agarose solution, placed on MS solid medium (0.8% agarose), and then grown in a growth chamber at 22°C with 40% humidity and continuous white light ($50 \mu\text{mol s}^{-1} \text{m}^{-2}$) for 14 d. To analyze the expression of *FOA1* in response to ABA and NaCl, 14-day-old wild-type Col-0 seedlings were treated with ABA or NaCl by immersing their roots in liquid MS medium containing ABA ($10 \mu\text{mol L}^{-1}$) or NaCl (100 mmol L^{-1}) for 0, 1, 2, 4, 6 and 8 h, respectively. To analyze the expression of ABA and stress-response genes in the wild-type Col-0 and *foa1* mutant in response to ABA, the 14-day-old seedlings were placed in liquid MS medium with ABA ($100 \mu\text{mol L}^{-1}$) for 0 or 6 h. Samples were collected, frozen in liquid nitrogen, and stored at −80°C for RNA isolation and gene expression analysis.

Seed germination analysis was as follows: Seeds were suspended in 0.1% agarose solution, placed on MS solid medium (0.8% agarose) supplemented with different concentrations of ABA (0, 0.3, $0.6 \mu\text{mol L}^{-1}$), and then grown in a temperature controlled chamber for 5 d, after which the germination rate was scored. Each value was obtained from three independent experiments using approximately 100 seeds each.

Root elongation assay was as follows: Seeds were suspended in 0.1% agarose, placed on MS solid medium for 3 d, then transferred to MS solid culture medium (0.8% agarose) with different concentrations of ABA (0, 5, $10 \mu\text{mol L}^{-1}$) and grown vertically. Six days later, the root length was measured. Each value was obtained from three independent experiments with 20 samples each.

1.4 Stomatal aperture measurement

Stomatal apertures were measured according to the procedure described by Pei [24]. Plant rosette leaves of a 4 to 5-week-old wild-type, T-DNA insertion mutants and

over-expression lines during the same growth stage were collected, and dipped into stomatal-opening solution (10 mmol L⁻¹ KCl, 7.5 mmol L⁻¹ iminodiacetic acid, 10 mmol L⁻¹ MES, and 10 mmol L⁻¹ Tris-HCl, pH 6.2) for 10 h to induce a fully stomatal opening. They were then immersed for another 2 h in the same solution with different concentrations of ABA (0, 1, 10 μmol L⁻¹). Stomatal apertures were photographed and measured under a microscope (NICON TE2000). Each experiment was repeated three times.

1.5 Assessment of the water-loss rate

The water-loss rate was measured as described by Shan [25]. Four fully expanded rosette leaves were collected from an approximately 3-week-old wild-type, T-DNA insertion mutants and over-expression transgenic plants. After positioning them onto clean filter paper, the detached leaves were placed into a temperature controlled growth chamber at 25°C with 60% humidity. Fresh weight was recorded at 30 min intervals and the water-loss rate was calculated using the fresh-weight loss of the detached leaves. Each experiment was repeated three times.

1.6 Proline content measurement

Wild-type *Arabidopsis*, T-DNA insertion mutants and over-expression lines were treated with ABA for 3 d by spraying 0 or 100 μmol L⁻¹ ABA solution on the leaves once a day. Rosette leaves were collected and proline was extracted using the sulfosalicylic acid method [26]. The proline content was measured using the absorbance at 520 nm. Each experiment was repeated three times.

1.7 Total RNA isolation and RT-PCR analysis

Total RNA was isolated using an RNA easy Mini Kit (Ambiogen Biological Technology) according to the manufacturer's instructions. First strand cDNA was synthesized using the Maxima[®] First cDNA Synthesis Kit (Fermentas) according to the manufacturer's specification. The cDNA product was diluted for 10-fold, and 1 μL of diluted cDNA was used in a 20 μL PCR reaction. The DNA sequences of the RT-PCR primers used in this study are listed in Table 2.

Table 1 Primer sequences used for cloning the *FOA1* coding sequence and identifying homozygous lines of T-DNA insertion mutants

Primer name	Primer sequence 5'-3'
<i>FOA1</i>	F: CAAAAAAGCAGGCTTCATGACGAAAATTTC CGATCTT R: CAAGAAAGCTGGGTCCTAGATTTGCACAGA ACTTGGGA
R0	ATTTTGCCGATTTTCGGAAC
CS857416	F: CAGAAACTCTTCTCGTGGTG R: AAGAAAAAGCCGAAGCAAAAG

Table 2 Primer sequences used for RT-PCR

Primer name	Primer sequence 5'-3'
<i>FOA1</i>	F: GCCTTGTTGTTGATGATACCATA R: GATTTCACAGAACTTGGAACAT
<i>ACTIN2</i>	F: CACTGTGCCAATCTACGAGGGT R: CACAAACGAGGGCTGGAACAAG

PCR was generally performed with a 5 min denaturation at 95°C, followed by 26 or 32 cycles consisting of the following steps: 95°C for 30 s, 58°C for 30 s, 72°C for 30 s for the *ACTIN2* and *FOA1* genes. The PCR products were separated on 1.5% agarose gels. The *ACTIN2* gene was used as the internal control. RT-PCR reactions for each experiment were repeated at least three times.

1.8 Real-time fluorescence quantitative PCR

The cDNA product was diluted 20-fold, and 2 μL of diluted cDNA was used in a 20-μL PCR reaction. The PCR was performed using a SYBR[®] Green I kit (TOYOBO, Japan) in an Mx3000P PCR machine (Stratagene, USA). The reaction started with a denaturation stage at 95°C for 10 min, which was then followed by 40 cycles, each cycle composed of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s. The experiment was repeated three times from three independent experiments. The *ACTIN2* gene was used as an internal control. The relative expression level was analyzed by the Mx3000P software. The sequences of the real-time fluorescence quantitative PCR primers used in this study are listed in Table 3.

2 Results

2.1 Expression of *FOA1* in different organs of *Arabidopsis*

The expression patterns of a gene in specific organs usually

Table 3 Primer sequences used for real-time fluorescence quantitative PCR

Primer name	Primer sequence 5'-3'
<i>FOA1</i>	F: GCTTACATCGTTGGAGAAG R: GCACAGAACTTGGAACATAA
<i>ABF3</i>	F: AATGGCGGATTCTATGGATT R: GCATCTGTAGTGGCTGAG
<i>OST1</i>	F: GGATCAACCGGGCCAAAG R: TGAGTGCTGCAGGAGGAA
<i>ABI1</i>	F: AGCTGCTGATATAGTCGTCGTTGATA R: GAGGATCAAACCGACCATCTAACA
<i>RD22</i>	F: GACCATTGAGGAGTGTGAA R: TCGGTGCGTTCTTCTTAG
<i>RD29A</i>	F: AGGAACCACCACTCAACA R: GCTCGTCATCATCATCT
<i>RD29B</i>	F: AAGGAGACGCAACAAGGG R: ACGGTGGTGCCAAGTGAT
<i>ACTIN2</i>	F: CACTGTGCCAATCTACGAGGGT R: CACAAACGAGGGCTGGAACAAG

provide clues to its biological functions. To analyze the expression of *FOA1* in different organs of *Arabidopsis*, roots, stems, cauline leaves, flowers and petioles were collected separately from the wild type (Col-0) during the flowering period. Total RNA was isolated and used in real-time fluorescence quantitative PCR analysis. The results showed that *FOA1* is expressed in all the tested organs, and the relative expression level is higher in roots and flowers and lower in the stem (Figure 1), indicating that *FOA1* might play a role in plant root and flower development.

2.2 Expression of *FOA1* is induced by exogenous ABA and NaCl

There is an ABRE *cis*-element in the promoter sequence of *FOA1* gene, thus the expression of *FOA1* in response to ABA and NaCl was analyzed. Real-time fluorescence quantitative PCR analysis showed that ABA and NaCl induced the expression of *FOA1* rapidly, peaking at 4 and 2 h, respectively. *FOA1* expression decreased with the treatment time (Figure 2), suggesting that *FOA1* might be an ABA signaling-related gene, and is involved in the plant stress response.

2.3 Molecular identification of a T-DNA insertion mutant and *FOA1* over-expression lines

To further study the biological function of *FOA1* in ABA

signaling, T-DNA insertion mutant *CS857416* was purchased from the ABRC. The sequence of the T-DNA flanking region showed that the insertion was located 599 bp downstream of the ATG start codon (Figure 3A). Homozygous individuals were identified by PCR with primers R, F and R0, and four individuals were identified to be homozygote and named *foa1* (Figure 3B). The expression of *FOA1* in *foa1* mutant was analyzed by semi-quantitative RT-PCR. The result showed that the *FOA1* signal in the wild type was strong, but the corresponding signal in the *foa1* mutant was barely detectable (Figure 3C), indicating that the T-DNA insertion severely impaired *FOA1* expression.

In addition, the *FOA1* coding sequence was subcloned into vector pEarleyGate 203 (N-Myc) downstream of the CaMV35S promoter, and introduced into *Arabidopsis* mutant *sgs* (subsequently, *sgs* refers to the *sgs* mutant lines expressing wild-type levels of *FOA1* in this study). Homozygous transgenic lines were identified by Basta resistant selection and real-time fluorescence quantitative PCR analysis. As shown in Figure 3D, *FOA1* is overexpressed in *FOA1ox1* and *FOA1ox2* transgenic lines, and its expression level is about 5 and 25 times higher, respectively, compared with the wild-type *sgs*.

2.4 *FOA1* is involved in the ABA-mediated inhibition of seed germination and root elongation

To elucidate the role of *FOA1* in ABA signaling, seeds of the wild type, the *foa1* mutant, and over-expression lines *FOA1ox1* and *FOA1ox2* were plated on MS solid medium with different concentrations of ABA. The germination rate was scored 5 days later. The *foa1* mutant displayed a reduced germination rate compared with wild-type Col-0 (Figure 4A). Seed germination was seriously inhibited in the presence of increasing concentrations of ABA, and decreased by 25% and 20% in *foa1* and Col-0, respectively, and 22%, 31%, and 36% in *FOA1ox1*, *FOA1ox2*, and *sgs*, respectively, in the presence of 0.6 $\mu\text{mol L}^{-1}$ ABA (Figure 4B), which indicated that *foa1* was more sensitive to ABA than Col-0, and the *FOA1ox1* and *FOA1ox2* were less sensitive to ABA compared with *sgs* in terms of seed germination.

Figure 1 Real-time fluorescence quantitative PCR analysis of *FOA1* expression in different organs of *Arabidopsis*. 1.0 symbolizes that the relative expression level of *FOA1* in the root is set as 1.

Figure 2 Real-time fluorescence quantitative PCR analysis of the expression of *FOA1* in response to exogenous ABA and NaCl. 1.0 symbolizes that the relative expression level of *FOA1* in the indicated time point is set as 1.

Figure 3 Molecular identifications of T-DNA insertion mutant and *FOA1* transgenic plants. A, Schematic of the *FOA1* gene and the T-DNA location in the T-DNA insertion mutant *CS857416*. B, Electrophoresis analysis for identifying homozygous individuals of the T-DNA insertion mutant by PCR. 1–4, 5, and M symbolize homozygous individuals of T-DNA insertion mutant, wild-type *Col-0*, and DNA marker, respectively. C, RT-PCR analysis of *FOA1* expression in *foa1* mutant. *ACTIN2* was used as an internal control. D, Real-time fluorescence quantitative PCR analysis of *FOA1* expression in *FOA1ox1* and *FOA1ox2* transgenic lines. 1.0 symbolizes that the relative expression level of *FOA1* in wild-type *sgs* is set as 1.

Similar to the seed germination assay results, *foa1* showed shorter main roots, but *FOA1ox1*, *FOA1ox2* showed longer main roots, and both showed an altered response to ABA in terms of root elongation (Figure 5). The roots of

foa1 were significantly more sensitive to ABA than wild-type *Col-0* in the presence of $10 \mu\text{mol L}^{-1}$ ABA, but the roots of *FOA1ox1* and *FOA1ox2* showed significantly reduced sensitivity in the presence of 5 and $10 \mu\text{mol L}^{-1}$ ABA compared with wild-type *sgs* (Figure 5). These results indicated that *FOA1* might play a negative role in ABA-mediated inhibition of root elongation and seed germination.

2.5 *FOA1* is involved in ABA-mediated control of stomatal closure and water loss

ABA is known to induce stomatal closure. To investigate whether *FOA1* is involved in ABA-mediated control of stomatal closure, leaves of the wild type, *foa1*, *FOA1ox1* and *FOA1ox2* were sampled and immersed in stomatal opening buffer for 10 h to induce full stomatal opening. They were then treated for another 2 h in the presence of different concentrations of ABA. The results showed that the stomatal aperture of *foa1* was larger than that of the wild type without ABA treatment ($0 \mu\text{mol L}^{-1}$ ABA)(Figure 6A). *foa1* exhibited hypersensitivity to ABA, and the ratio of the longitudinal to the traverse diameter of the stomata in *foa1* was lower than that in the wild-type *Col-0* in the presence of $10 \mu\text{mol L}^{-1}$ ABA (Figure 6B). By contrast, the stomatal

Figure 4 Seed germination of wild type, *foa1*, *FOA1ox1* and *FOA1ox2* in response to ABA. A, Pictures of seed germination of wild type, *foa1*, *FOA1ox1* and *FOA1ox2* grown on MS solid medium for 5 d. B, Germination rates of wild type, *foa1*, *FOA1ox1* and *FOA1ox2* scored when grown on MS solid medium for 5 d. * and ** indicate that the value of a *t*-test was $P < 0.05$ and $P < 0.01$ respectively, and indicate a significant difference between the wild type and mutant or overexpression lines.

apertures of *FOA1ox1* and *FOA1ox2* were smaller than wild-type *sgs* without ABA treatment (Figure 6A). In the presence of $10 \mu\text{mol L}^{-1}$ ABA, the longitudinal to traverse diameter ratio of stomata in wild-type *sgs* decreased significantly, but was almost unchanged in *FOA1ox1* and *FOA1ox2* (Figure 6B). These results indicated that *FOA1* might also play a negative role in ABA-regulated stomatal closure.

Water loss triggered by ABA through stomatal closure is a crucial survival mechanism for plants in response to drought stress [16,27]. To investigate the role of *FOA1* in regulating water loss, leaves of the wild type, *foa1*, *FOA1ox1* and *FOA1ox2* were collected and placed in a temperature controlled growth chamber. The weight of the

detached leaves was measured at 30 min intervals. Inconsistent with the stomatal aperture results, the water-loss rate of *foa1* was about 1.5 times higher than that of wild-type Col-0. The water-loss rate of *FOA1ox1* and *FOA1ox2* was about 1.5–2 times lower than that of wild-type *sgs* (Figure 7), which might be due to the larger stomatal aperture of *foa1* compared with wild-type Col-0, and the smaller stomatal aperture of *FOA1ox1* and *FOA1ox2* compared with wild-type *sgs* before ABA treatment (Figure 6).

2.6 FOA1 is involved in ABA's effect on proline accumulation in plants

Exogenous ABA treatment leads to proline accumulation in

Figure 5 Root lengths of wild type, *foa1*, *FOA1ox1* and *FOA1ox2* in response to ABA. Data represent the mean root length of at least 20 seedlings. * and ** indicate that the value of a *t*-test was $P<0.05$ and $P<0.01$ respectively, and indicate a significant difference between the wild type and the mutant or overexpression lines.

Figure 6 Stomatal aperture of the wild type, *foa1*, *FOA1ox1* and *FOA1ox2* in response to ABA. A, Pictures of stomata of the wild type, *foa1*, *FOA1ox1* and *FOA1ox2* in the presence of different concentrations of ABA. B, Data are expressed as the ratio of the longitudinal diameter to the traverse diameter (L/T) of stomata. * and ** indicate that the value of a *t*-test was $P<0.05$ and $P<0.01$ respectively, and indicate a significant difference between the wild type and the mutant or overexpression lines.

plants, including barley, maize, rice, soybean and *Arabidopsis* [28]. To investigate whether *FOA1* affects proline accumulation in plants in response to ABA, the proline contents of the wild type, *foa1*, *FOA1ox1*, and *FOA1ox2* plants in response to ABA were determined. As shown in Figure 8, proline accumulated in plants in the presence of ABA, but there were significant differences between the wild type and the mutants. Proline content increased by 1.2 and 1 times in *foa1* mutant and wild-type Col-0 respectively, and increased by 0.7 and 1.6 times in *FOA1* overexpression lines and wild-type *sgs* respectively (Figure 8). These results indicated that *foa1* accumulated significantly more proline than wild-type Col-0, while *FOA1ox1* and *FOA1ox2* accumulated significantly less proline than wild-type *sgs*. Thus, *FOA1* might also play a negative regulatory role in ABA's control of proline accumulation.

2.7 *FOA1* regulates the expression of ABA and stress-responsive genes

To provide molecular evidence of *FOA1*'s involvement in ABA signal transduction, the expressions of a series of ABA-, drought-, and salt stress-related genes were analyzed in the wild type and *foa1* mutant treated with or without ABA. The genes included transcription factors *ABI1* [29] and *ABF3* [30], *ABF3* interaction protein kinase *OST1* [31],

and ABA and stress-responsive genes *RD29A*, *RD29B* and *RD22* [32,55]. The results showed that the expressions of all tested genes were induced by ABA, but there were differences between *foa1* and the wild-type Col-0 (Figure 9). The expression levels of *ABI1*, *OST1* and *ABF3* in *foa1* were higher, and the expression levels of *RD29A*, *RD29B* and *RD22* in *foa1* were lower than that in the wild-type Col-0 (Figure 9). These results indicated that a null mutation of *FOA1* could result in altered expression of ABA, drought, salt stress-responsive genes, which further supported the hypothesis that *FOA1* is an ABA signaling-related gene, which might play a role in stress resistance of plants through the ABA signaling pathways.

3 Discussion

As members of the SCF complex, F-box proteins control many hormone signal transductions, including GA [7], auxin [9,10], and ethylene signal transduction by identifying target proteins and mediating their degradation. Recently, certain F-box proteins were also found to be involved in ABA signal transduction [34]. F-box protein EDL3 functions as a positive regulator in ABA signal transduction and regulates seed germination, root growth, etiolated seedlings turning green, and flowering [8]. TLP9 may be involved in

Figure 7 Water loss of the wild type, *foa1*, *FOA1ox1* and *FOA1ox2* in response to ABA. Water loss is expressed as a percentage of the initial fresh weight at indicated intervals. FW, fresh weight.

Figure 8 Proline accumulation of wild type, *foa1*, *FOA1ox1* and *FOA1ox2* in response to ABA. FW, fresh weight. * and ** indicate that the value of a T test was $P < 0.05$, and $P < 0.01$ respectively, and indicate a significant difference between the wild type and the mutant or overexpression lines.

Figure 9 Expressions of ABA and stress-responsive genes in wild-type Col-0 and *foa1* mutant in response to ABA. 14-day-old wild-type and *foa1* seedlings were treated with 100 $\mu\text{mol L}^{-1}$ ABA for 0 or 6 h, and sampled for RNA isolation. The expressions of ABA and stress responsive genes were analyzed by real-time fluorescence quantitative PCR. *, **, and *** indicate that the value of a *t*-test was $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, and indicate significant differences between the wild type and the mutant. 1.0 symbolizes that the relative expression level of *FOA1* in Col-0 without ABA treatment is set as 1.

ABA-mediated reactions by identifying negative regulators of ABA signal transduction. The *TLP9* mutant showed loss of sensitivity to ABA and *TLP9* over-expression lines showed hypersensitivity to ABA [35]. A null mutation of *DOR* leads to stomatal closure and enhanced drought-resistance. By comparison, overexpression of *DOR* in *Arabidopsis* resulted in hypersensitivity to drought stress. These results indicated that *DOR* plays a negative role in response to drought stress through ABA signal transduction [16]. In this study, we reported that *FOA1* is an ABA signaling-related gene, involved in many ABA-regulated *Arabidopsis* biological processes, including seed germination, root elongation, stomatal closure, water-loss rate and proline accumulation.

The expression pattern of the F-box gene in different organs was analyzed. *FOA1* is expressed in all tested organs, and showed the highest transcript level in roots and flowers (Figure 1). Phenotype analysis showed that the *FOA1* null mutant *foa1* has a shorter root phenotype, while the *FOA1* over-expression lines *FOA1ox1* and *FOA1ox2* exhibited a longer root phenotype (Figure 5), which is consistent with the organ expression result, and indicated that *FOA1* might be involved in plant root development. No flower phenotype was found in the *foa1* mutant or in the *FOA1ox1* and *FOA1ox2* overexpression lines in this study.

There is an ABRE element in the promoter region of *FOA1*, and the expression analysis results proved that exogenous ABA and NaCl rapidly induced the transcription of *FOA1*, which indicated that *FOA1* is probably an ABA signaling-related gene. Phenotype analysis showed that the

FOA1 null mutant *foa1* exhibited increased sensitivity and *FOA1ox1* and *FOA1ox2* overexpression lines exhibited decreased sensitivity to ABA in the seed germination, root elongation, stomatal closure assays compared with the wild type. *foa1* accumulated significantly more proline than wild type Col-0, while *FOA1ox1* and *FOA1ox2* accumulated significantly less proline than the wild-type *sgs* in the presence of ABA. These results suggested that *FOA1* plays a negative role in ABA signal transduction. The proline content of plants is a characteristic feature of stress resistance [28]. Thus, our results suggested that *FOA1* is involved in ABA-dependent stress resistance. Differences between Col-0 and *sgs* in response to ABA were also discovered in this study (Figures 4–6), which may be a result of the recessive monogenic mutation carried by the *sgs* mutant [23].

The expression analysis of stress-responsive genes showed that *ABF3*, which is a positive regulatory transcription factor in ABA signal pathway [30,36], is expressed higher in the *foa1* mutant than in the wild-type Col-0. *OST1*, which activates *ABF3* and is activated by ABA [32], is also expressed higher in the *foa1* mutant than in the wild type, suggesting that ABA signal transduction is enhanced in the *foa1* null mutant, which was consistent with the phenotype of *foa1* (hypersensitivity to ABA). *ABI1* is a group A PP2C in *Arabidopsis* and has been described as a negative regulator of ABA signaling. *ABI1* is strongly induced by ABA [8,29,36,37]; its transcription level increased in lines over-expressing *ABF3*, *ABF4* [30], or *TSK1* [36] in which ABA signaling is enhanced, while it decreased in ABA-insensi-

tive mutants such as *abi5-1* and *snrk2.2/snrk2.3/snrk2.6* triple mutant, in which ABA signaling is blocked [38]. Consistent with previous results, the expression of *ABI1* increased in the ABA hypersensitive mutant *foal1*. The expressions of *RD29A*, *RD29B* and *RD22* are strongly induced by salt, drought, cold and ABA [32,33]. Similar results were obtained in this study; the transcript levels of *RD29A*, *RD29B*, and *RD22* increased in both the wild type and *foal1* in response to ABA. However, their expression levels in *foal1* were lower than in the wild-type Col-0. These results suggested that a null mutation of *FOA1* resulted in altered transcript levels of ABA and stress-responsive genes, providing further evidence that *FOA1* is an ABA signaling-related gene, and might be involved in the plant stress response.

The FOA1 protein carries an F-box domain and is thus presumed to act as a component of the SCF complex. We believe it is involved in ABA signal transduction by targeting transcription factors that regulate the expression of ABA and stress-responsive genes for degradation in the proteasome. This hypothesis requires to be further tested.

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